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RESEARCH ARTICLE

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Prospective evaluation of a screening algorithm for carbapenemase-producing *Enterobacteriaceae*

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Abstract

Background: Carbapenemase-producing *Enterobacteriaceae* (CPE) have become a major public health issue. The objective of the present study was to prospectively assess the analytical performance of a CPE detection algorithm based on phenotypic tests (the screening test) and MALDI-ToF hydrolysis (the confirmatory test).

Methods: Over a 6-month period and based on a disk diffusion method, 74 carbapenem-resistant strains were included in this study.

Results: Of the collected isolates, 54 turned out to be negative after phenotypic tests. Hence, 20 strains (including all of the CPEs) were checked with the confirmation test. Seven strains were positive. After molecular biology assessments in a reference center, three of the seven were found to be false positives. The algorithm had a negative predictive value and a sensitivity of 100%, a specificity of 77%, and a positive predictive value of 20%.

Conclusion: The algorithm has a 24-hour turnaround time and helps to avoid using expensive molecular biology tests; we consider that it can be used on a routine basis for screening clinical strains.

KEYWORDS

carbapenem, carbapenemase-producing *Enterobacteriaceae*, prospective, resistance, screening algorithm

1 | INTRODUCTION

Over the past few decades, the effectiveness of carbapenem drugs has been progressively compromised by the worldwide emergence of resistant Gram-negative isolates, including *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*.[1] In the *Enterobacteriaceae*, carbapenem resistance may be related to (a) the combination of a decrease in bacterial outer membrane permeability and the overexpression of β-lactamases with no carbapenemase activity or (b) carbapenemase expression.[2,3] Given that only a very small proportion (0.08%) *Enterobacteriaceae* produce carbapenemase, the first of these two mechanisms is most common in carbapenem nonsusceptible *Enterobacteriaceae* (NSE).[4] Therefore, detecting carbapenemase-producing *Enterobacteriaceae* (CPE) among carbapenem NSE strains is difficult. However, screening for carbapenem NSE strains is essential because of their propensity to spread easily through horizontal gene transfer (plasmids, transposons).[2]

A variety of commercially available and in-house phenotypic and molecular laboratory tests have been used to detect carbapenemases.[5] Most are used as additional tests after antimicrobial susceptibility has been performed. Furthermore, carbapenemase screening assays are expensive and have to be performed by experienced personnel. Routine use of these assays is not possible in all clinical laboratories.

Due to the worldwide spread of CPE, carbapenemase detection is now a critically important tool for indicating the most appropriate treatment. Therefore, the French National Reference Centre for Antimicrobial Resistance (NRC-AR),[6] the *Comité de l'Antibiogramme*

de la Société Française de Microbiologie (CA-SFM), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[7] recently proposed algorithms that combine phenotypic, hydrolysis-based, and molecular tests.

The objectives of the present study were to develop a CPE detection algorithm that matched the NRC-AR/CA-SFM/EUCAST guidelines[6,7] and then evaluate it prospectively for six months. Indeed, we found that the routine use of this algorithm enabled rapid, efficient, and cost-effective CPE detection.

2 | MATERIALS AND METHODS

From February to July 2016, we assessed all clinical enterobacterial strains with reduced susceptibility to carbapenems (*ie*, diameter zone <28 mm for ertapenem or <24 mm for imipenem, respectively, together with a diameter zone <15 mm for ticarcillin-clavulanate) recovered in our laboratory or sent from external laboratories. Strains obtained from screening rectal swabs were not included in the study. Bacterial species were identified by using MALDI-ToF mass spectrometry (MALDI Biotyper; Bruker Daltonics, France). Susceptibility testing (using the agar diffusion method) was performed in line with the CA-SFM 2013 guidelines[8] on Mueller-Hinton (MH) agar medium (Bio-Rad, Marne-la-Coquette, France). For CPE detection, a temocillin disk was added to each MH agar plate, in accordance with the CA-FSM 2015 guidelines.[7] The presence of extended-spectrum β-lactamases (ESBLs) was determined by highlighting a synergy image between expanded-spectrum cephalosporins and clavulanic acid on agar plates and cloxacillin-containing agar plates (Bio-Rad). Because cloxacillin is known to inhibit AmpC-type β-lactamases, the overproduction of AmpC was evidenced using the cefoxitin-cloxacillin double-disk synergy method (CC-DDS) as described previously.[9] The MICs of imipenem and ertapenem were determined with Etest strips (bioMérieux, Marcy-l'Etoile, France),

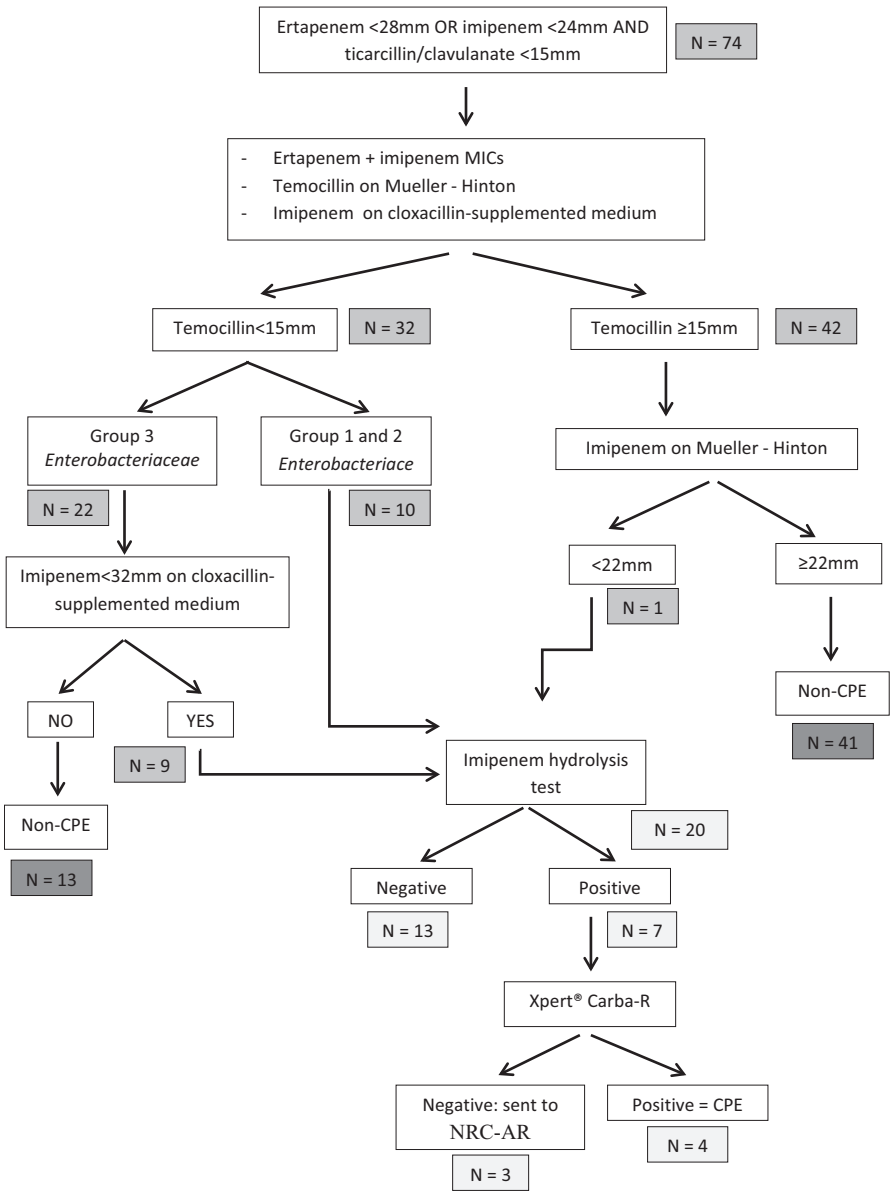

FIGURE 1 Results of the screening algorithm for CPE detection (n = 74). Group 1 *Enterobacteriaceae*: nonchromosomic -lactamase producers; Group 2 *Enterobacteriaceae*: groups 2a, 2b, and 2be of Bush functional classification of -lactamase producers[14]; and Group 3 *Enterobacteriaceae*: group 1 of Bush functional classification of -lactamase producers[14]

TABLE 1 Characteristics of the 74 clinical enterobacterial strains used to test the screening algorithm

	Non-CPE isolates (porin-deficient)			CPE isolates		
Species	**ESBL**	**Cephalosporinase**	**Oxacillinase**	**OXA-48**	**NDM**	**Total**
E. cloacae	12	36	-	-	-	48
K. pneumonia	10	3	-	3	1	17
E. aerogenes	2	5	-	-	-	7
E. coli	1	-	1	-	-	2
Total	25	44	1	3	1	74

according to the CA-SFM 2013 guidelines.[8] All of the isolates were genetically tested for the presence of carbapenemases using a real-time PCR assay (Xpert Carba-R version 2; Cepheid, Sunnyvale, CA, USA), according to the manufacturer's instructions. This assay includes targets for the most common carbapenemases worldwide: bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, bla_{OXA-48}, and $bla_{OXA-181}$.

The collected strains are screened with the algorithm illustrated in Figure 1. The algorithm comprises three steps: a phenotypic test (for screening), a hydrolysis test (for confirmation), and a molecular test (for characterization). Firstly, diameter zones of ertapenem, imipenem, and ticarcillin-clavulanate were selected to detect carbapenem NSE that matched the experts' recommendations.[6,7] Next, in order to increase our algorithm's specificity, the MICs of imipenem and ertapenem were determined, temocillin was tested on MH agar plates, and imipenem was tested on MH agar containing 250 mg/L cloxacillin.[10] Secondly, strains requiring additional analyses for CPE detection after the phenotypic test underwent a MALDI-ToF-based imipenem hydrolysis test, as described previously.[11] Thirdly, MALDI-ToF-positive strains were characterized with the Xpert Carba-R test. When it was negative, the strain was sent to the NRC-AR for assessment.

3 | RESULTS

A total of 74 nonduplicate carbapenem NSE from clinical isolates were collected between February and July 2016 (Table 1). Strains were mostly obtained from urinary, respiratory, abdominal, and blood cultures samples. All had a diameter zone <28 mm for ertapenem or <24 mm for imipenem, respectively, together with a diameter zone <15 mm for ticarcillin-clavulanate. MALDI-ToF identification revealed 48 *E cloacae*, 17 *K pneumoniae*, 7 *E aerogenes*, and 2 *E coli* strains.

The Xpert Carba-R test was negative for 70 of 74 strains; they were considered as the non-CPE strains. In total, 44 of them produced cephalosporinase (36 *E cloacae*, 5 *E aerogenes*, and 3 *K pneumoniae*), 25 produced ESBL (12 *E cloacae*, 10 *K pneumoniae*, 2 *E aerogenes*, and 1 *E coli*) and 1 *E coli* produced narrow-spectrum oxacillinase (Table 1). All four CPE (Xpert Carba-R positive) were *K pneumoniae* isolates, 3 produced OXA-48, and 1 produced NDM (Table 1). We applied our new screening algorithm to the 74 included strains (Figure 1). Fifty-four strains suspected of producing a carbapenemase were ruled out by the phenotypic test, and the remaining 20 required a hydrolysis test (Figure 1). Thirteen of these 20 strains were negative in the MALDI-ToF test and so were discarded and considered to be non-CPE strains. Four of seven MALDI-ToF-positive strains were positive with the Xpert Carba-R test (three OXA-48 producers and 1 NDM producer). The NRC-AR confirmed that the three PCR-negative strains were indeed carbapenemase-negative. In the absence of carbapenemase production, we consider that low susceptibility to carbapenems was due to low bacterial outer membrane permeability (elevated carbapenem MICs) associated with the overexpression of β-lactamases with no carbapenemase activity. When applied to the prospective collection of 74 carbapenem NSE strains, our algorithm had a negative predictive value (NPV) of 100%, a sensitivity of 100%, a specificity of 77%, and a positive predictive value (PPV) of 20% (Table 2).

4 | DISCUSSION

Given the emergence of carbapenem NSE strains, there is a critical need for a highly sensitive method that can clearly discriminate between strains that do not produce carbapenemase and those that require complementary testing. The screening algorithm developed here complies with the latest guidelines[6,7] and can be easily implemented on a routine basis by using the disk diffusion method for susceptibility testing. In fact, the algorithm is based on phenotypic tests (MICs and diameter zones for the most commonly tested antibiotics, including temocillin) and then an in-house MALDI-ToF hydrolysis test. These tests are cost-effective and do not require experienced staff. Use of this algorithm enabled us to rule out carbapenemase production (with a NPV of 100% and a sensitivity of 100%) in 54 of the 74 strains (73%) after the first step in the test (ie, with no need for additional testing). The use of this type of algorithm might save time and money for

TABLE 2 Distribution of the 74 strains after categorization with the CPE detection algorithm

	CPE	Non-CPE	Total
Additional tests required	4	16	20
No additional tests required	0	54	54
Total	4	70	74

clinical microbiology laboratories faced with a similar epidemiological situation; indeed, additional testing might be necessary in less than one third of the cases (74 strains collected, 20 subjected to additional testing, 7 in real-time PCR assays, and only three sent to the NRC-AR for detailed assessment). One of the strengths of the present study was its prospective collection, which enables us to test the algorithm with our local strains. Between February and July 2016, 3516 samples of *Enterobacteriaceae* were collected in our laboratory. Of these, 74 of them were carbapenem NSE (2.1%), including 4 CPE (0.11%). These local frequencies are slightly higher than the values determined for France as a whole (0.6% for carbapenem NSE and 0.08% for CPE).[4,12] The NRC-AR has evaluated a similar algorithm that did not include imipenem disks on cloxacillin-containing MH agar.[13] About one third of the collected strains were identified as noncarbapenemase-producers, in the absence of any additional testing. However, this evaluation was based on the strains sent to the NRC-AR for assessment (34.8% CPE), which do not reflect the epidemiological profile in France as a whole.[13] In view of our local epidemiology and our prospective collection, one limitation of this study relates to the low proportion of CPE (mainly OXA-48, rather than less common carbapenemases such as GES-type enzymes). This low proportion might explain our algorithm's relatively low PPV. In fact, the PPV of confirmatory tests would probably be greater when the proportion of CPE is higher.[10] Lastly, we used the EUCAST cutoffs for CPE detection because they gave better results than the Clinical and Laboratory Standards Institute's clinical breakpoints.[5] As suggested by Dortet *et al*,[13] the higher prevalence of OXA-48 producers in European countries (relative to the USA) might explain this difference.

In conclusion, the CPE screening algorithm assessed in the present study complied with expert guidelines and was designed to match our routine procedures and the local epidemiology. The algorithm combines phenotypic, hydrolysis, and molecular tests for the efficient detection of CPE. This makes it possible to rule out carbapenemase production in most carbapenem NSE (thanks to a high NPV) and limits the use of expensive, additional tests. A strategy based on the wide-ranging, rapid detection of carbapenemase producers may have a significant impact on preventing the spread of these pathogens in the community.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

ETHICAL APPROVAL

Not required.

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